SCALE UP STUDY AND COMPARISON OF THREE DOWNSTREAM PROCESSES OF MONOCLONAL ANTIBODY PRODUCTION USING SUPERPRO DESIGNER

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ABSTRACT

The main purpose of this study is to simulate the large scale production of monoclonal antibody (mAb) by using SuperPro Designer (SPD). Since the first discovery of mAb at 1970s, this type of antibody had become the most rapidly growing class of pharmaceutical. Problem with mAb production is the high cost and low amount of production but high demand for this therapeutic. In order to overcome this problem, large scale production had become one of the top priorities for mAb production. Large scale simulation study by using SPD can minimize time and cost production by eliminate the high cost of trial-and-error steps, as well as to find and simulate the optimization process for mAb production. In order to achieve the objective of this research, study is conducted in two steps which are simulation on the upstream and downstream process. For the upstream process, the stoichiometric equation is constructed base on the laboratory data and used to simulate the large volume of fermenter. For a downstream process, three flow of downstream process from different source a built, simulated and compared to choose the best process for purification of mAb. The result shows that the upstream process for 20000 L fermenter produced 0.00510 kg/Batch of mAb with concentration of 3.8×10^{-4} g/L (5.1103 g/Batch). Compared to the flow from SPD source and journal (S. Sommerfeld and J. Strube, 2005), the best downstream process was the flow from Inno Biologics Sdn.Bhd that yield 81% of mAb with a concentration of 4.14328 g/Batch. As a conclusion, the simulation of large scale production of mAb by using SPD are flow work for 20000 L of fermenter as upstream process and Inno Biologics Sdn. Bhd. flow work as downstream process.

ABSTRAK

Objektif utama dalam kajian ini adalah untuk simulasi skala besar pengeluaran antibodi monoklonal (mAb) dengan menggunakan SuperPro Designer (SPD). Sejak pertama kali mAb di temui pada tahun 1970-an, jenis antibodi ini telah berkembang pesat untuk industri farmasi. Masalah yang dihadapi dalam pengeluaran mAb adalah kos yang tinggi dengan jumlah pengeluaran yang rendah tetapi mempunyai permintaan yang tinggi. Untuk mengatasi masalah ini, pengeluaran berskala besar telah menjadi salah satu keutamaan untuk pengeluaran mAb. Simulasi skala besar dengan menggunakan SPD boleh meminimumkan masa dan kos pengeluaran dengan mengurangkan kaedah cuba jaya yang ber kos tinggi, serta mencari proses optimum bagi pengeluaran mAb. Untuk mencapai objektif, kajian dilakukan dalam dua langkah iaitu simulasi untuk proses huluan dan hiliran. Untuk proses huluan, persamaan stoikiometri dibina berdasarkan data makmal dan digunakan untuk simulasi pada fermenter yang lebih besar. Untuk proses hiliran, tiga aliran proses hiliran dari sumber yang berbeza dibina, disimulasi dan dibandingkan untuk memilih proses yang terbaik untuk penulenan mAb. Proses huluan untuk 20000 L fermenter menghasilkan 0.00510 kg / Batch mAb dengan kepekatan $3{,}8 \times 10^{-4}$ g / L (5,1103 g / Batch). Jika sumber SPD dan jurnal (S. Sommerfeld dan J. Strube, 2005) dibandingkan dengan Inno Biologics Sdn. Bhd, proses hiliran terbaik adalah proses dari Inno Biologics Sdn. Bhd dengan 81% daripada mAb asal diperolehi dengan kepekatan 4.14328 g / Batch. Kesimpulan yang boleh dibuat dari kajian ini adalah simulasi pengeluaran berskala besar mAb dengan menggunakan SPD ialah proses fermentasi 20000 L fermenter sebagai proses huluan dan proses Inno Biologics Sdn. Bhd sebagai proses hiliran.

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LIST OF ABBREVIATIONS

AEX	-	Anion-exchange chromatography
Amm	-	Ammonia
CAH	-	Congenital Adrenal Hyperplasia
CEX	-	Cation-exchange chromatography
Gln	-	Glutamine
Glu	-	Glucose
HIC	-	Hydrophobic chromatography
KCl	-	Potassium Chloride
KH_2PO_4	-	Potassium Phosphate Anhydrous
Lac	-	Lactate
mAb	-	Monoclonal antibody
Na_2HPO_4	-	Sodium Phosphate
RIPP	-	Recovery, Isolation, Purification, Polishing
SPD	-	SuperPro Designer
UF/DF	-	Ultrafiltration/Diafiltration
WFI	-	Water for Injection

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Monoclonal antibody (mAb or moAb) is a class of antibody that produced by a single type of immune cell that are all clones of a single parent cell that will recognizes a specific antigenic target. The term monoclonal implies antibody produced by a cell clone derived from a fusion of one antibody producing cell with one myeloma cell capable of growing indefinitely in culture (I. Y. Abdel-Ghany *et al*., 2009). Since from the first discovery of this technology at 1970s, advances in technology had produce high quality of mAb and led the development of excellent therapeutic agents that impact the human health. Up until 2009, there are four main type of mAb that is mouse (murine), humanized, chimeric and human (E. B. Rodrigues *et al*., 2009).

Since the production of mAb by hybridoma technology, mAb is currently used in many applications like the diagnosis and treatment of many diseases or standard binding protein for purification of substances (L. Legazpi *et al*., 2005). One of the applications of mAb is treatment for human intoxication like ciguatera seafood poisoning. Ciguatoxins are causative toxin for ciguatera is produced by the marine dinoflagellate *Gambierdiscus toxicus* and accumulated in various kinds of reef fish (T. Takeshi *et at*., 2009)

Hybridomas are hybrid between myeloma cell and mammalian cell that producing antibody (B-lymphocytes) (M. Butler, 2004). Myeloma is a tumor or cancerous cell that can replicate endlessly while B-lymphocytes are cell that can produce a single type of antibody (Prescott *et al*., 2005). When myeloma cells were fused with antibody-producing mammalian spleen cells, it was found that the resulting hybrid cells, or hybridomas, produced large amounts of mAb. This product of cell fusion combined the desired qualities of the two different types of cells: the ability to grow continually, and the ability to produce large amounts of pure antibody.

In the bioprocess, there is an upstream and downstream process for production of certain biological product like in this study, production of mAb. Upstream process is a first step in bioprocess which the biomolecules are grown, usually by bacteria or mammalian cell line in bioreactor (fermentation). It involved in cell line development, media optimization and cell culture optimization (Feng Li *et al*., 2005). When it reach the desired density, they are harvested and moved to downstream process of the bioprocess. In the downstream process, biological product will be purifying to meet purity and quality requirement. The downstream section can be divided into three parts: a capture section, a purification section and a polishing section (S. Sommerfeld and J. Strube, 2005).

Process simulators are offering opportunity to shorten or minimize the time and cost required for process development. Comparison of process alternatives on a consistent basis in simulation gave large number of process ideas to be synthesize and analyze interactively in a short time. SuperPro Designer (SPD) developed by Intelligent Inc. is software that suitable to simulate the bioprocess operation other than any simulation software like Aspen BPS that more towards chemical processes (S. A. Rouf *et al*., 2001). This package has the added advantage that it was specifically developed for simulation of biopharmaceutical process unit operations and processes and set up to capture the unique unit operational data requirements of biological processes (Ian Gosling, 2003).

1.2 Problem Statement

The production cost of mAb in industry is very high, it can achieve around US$ 100 to 1000 per gram of production cost (S. Sommerfeld and J. Strube, 2005). S.S Farid (2007) reported $660 to $1580/ft^2 and $1756 to $4220/L invested on antibody manufacturing site with total site capacities of 2000 L to 20000 L. Instead of that, low amount of mAb produced cannot afford the high demand of this rapidly growing therapeutics.

Because of the high production cost and low amount of product, the selling price becoming so high that ranges from US$5000 per gram for mAb to US$ 1 million for erythropoietin (S. K. W. Oh et al., 2004). To fulfill the high demand and lowering the cost mAb, large scale production of mAb had become one of the top priorities in biopharmaceutical industry.

Simulation can become one of the tools to minimize cost and time for production of mAb. Optimization can be made by firstly doing the simulation before undergo the large scale production and eliminate the trial-and-error step in biopharmaceutical process.

1.3 Objectives

The objective of this research is to study the upstream and downstream process of mAb from laboratory data base on SPD simulation. It is also to discover the potential for the large scale production of mAb by using the desired SPD.

1.4 Scope of the Study

Model for this study is hybridoma used to cultivate antibody towards Congenital Adrenal Hyperplasia (CAH), inherent disease that able to cause death within 14 days infant and abnormal sex. Scope in this study is to compare the simulation result with laboratory experiment data. The scope is also to build and simulate the large scale upstream process for mAb production by using the laboratory data. Other than that, the scope is to propose downstream process by build a several flow design for purification of mAb and analyze the best process base on yield and purity.

1.5 Rationale and Significance

This study has a potential to minimize cost and time for production of monoclonal antibody by doing the simulation first before furthering in clinical or large-scale production. Traditionally, process development of mammalian cell culture is based on trial-and-error experimentation. So, process simulation can overcome the trial-and error process development and facilitate rapid process improvement without extensive experimentation or disrupting existing operations.

CHAPTER 2

LITERATURE REVIEW

2.1 Upstream Process

In the bioreactor, cells survive, grow, die and produce mAb depending on their culture environment. Kinetic equation need to be developed to describe cell growth, nutrient consumption and product generation, the concentration of substrates and products is a factor for kinetics of hybridoma culture (L. Legazpi *et. al.*, 2005). In cell growth, glucose and glutamine are assumed to be the main substrate while ammonia and lactate produced from glucose and glutamine metabolism are assumed to be inhibitory to cell growth. Glutamine is also assumed as main limiting substrate for antibody production (J. D. Jae and J. P. Barford, 2000).

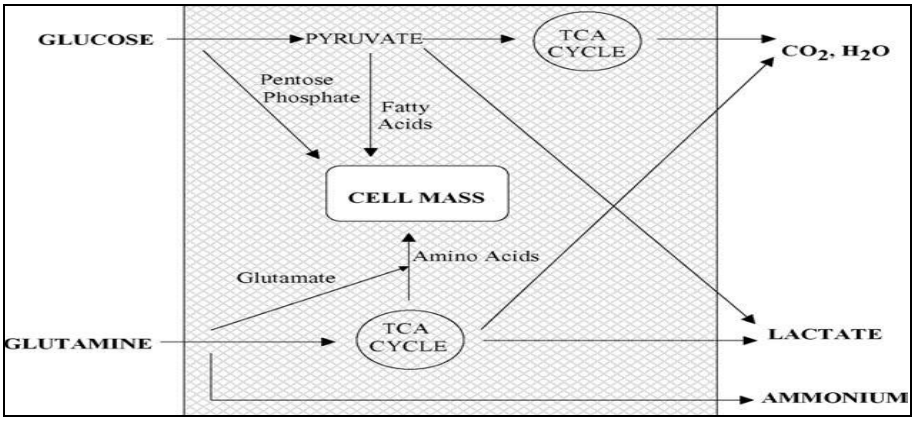

Figure 2.1: Main metabolic routes for hybridoma cells (L. Legazpi *et. al.*, 2005)

Specific of cell growth rate based on the concentration of key nutrient (glucose and glutamine) and the metabolites (lactate and ammonia) that follow monod kinetics. Cell death rate is based on the function of ammonia concentration that accumulates in the culture. For cell metabolism, concentration of nutrients and metabolites are compute by performing mass balance around bioreactor (C. Contoravdi *et. al*., 2007).

Model improvement over pre-existing model were developed by model development for batch and fed-batch operations were derived based on initial metabolic flux analysis(MFA) and the coefficient or parameters value for model equation were estimated by using quadratic programming(QP) and Metropolis-Hastings algorithm. Integrated model (combination between metabolites and biomass model) is capable of predicting concentration for substrates, extracellular metabolites, and viable and dead cell concentration (P. Dorka *et al*., 2009).

Many kinetic expressions and a large number of parameters are involved resulting in a complex identification problem. It is not possible to estimate simultaneously all parameters with the mathematical analysis, so strategy to narrow down the parameters involve should be taken by: firstly, estimate all parameters that could be analyzed independently, then the most insensitive parameters (mainly half-saturation constants-taken from literature), most sensitive parameters estimate by non-linear regression analysis, and finally improve model by tuning manually the most insensitive parameters (A. Teixeira *et al*., 2005).

From the study of A. C. Baughman *et al*. (2009) by taking the Gao *et al*. (2007) as case study, they state that half of generic metabolic reconstruction did not function significantly during major phase of the culture and that make the 32 reaction is reduced to 16 reactions. These 16 reactions then is further compacted by some technique which combine reactions that share common metabolites and reducing the reconstruction resulted in nine macro reaction.

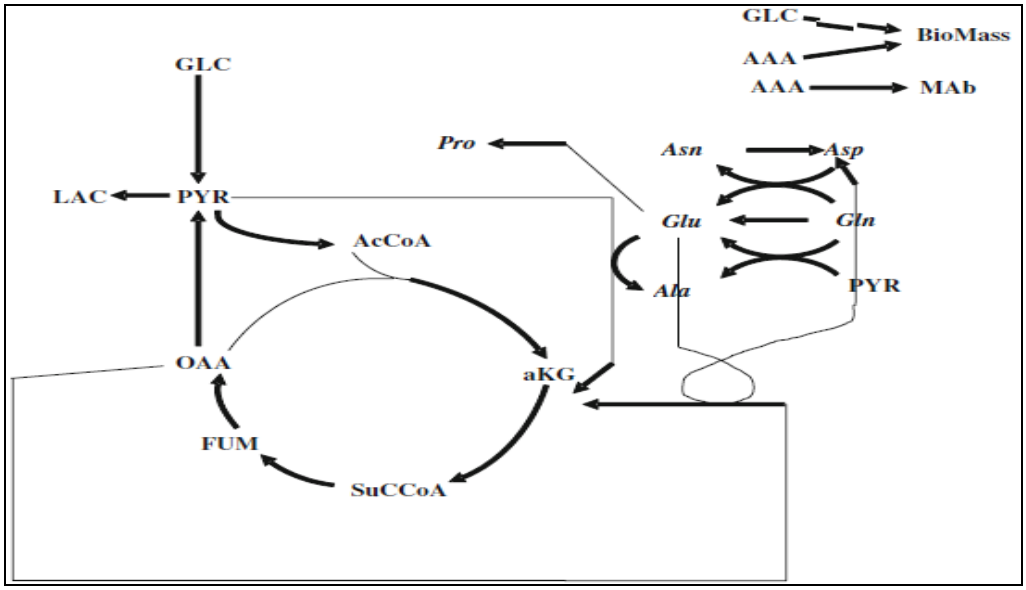

Figure 2.2: Reduced metabolic construction of hybridoma (A. C. Baughman *et al.*, 2009).

Table 2.1: The nine proposed macro-reactions (A. C. Baughman *et al.*, 2009).

Reaction 1 :	GLC ⟶ 2LAC
Reaction 2 :	GLC + 2GLU ⟶ 2ALA + 2LAC
Reaction 3 :	GLC + 2GLU ⟶ 2ASP + 2LAC
Reaction 4 :	GLU ⟶ PRO
Reaction 5 :	ASN ⟶ ASP + NH_3
Reaction 6 :	GLN + ASP ⟶ ASN + GLU
Reaction 7 :	0.0508GLC + 0.0577GLN + 0.006ASN + 0.0201ASP + 0.0016GLU + 0.0133ALA + 0.0081PRO ⟶ BM
Reaction 8 :	0.0104GLN + 0.0072ASN + 0.0082ASP + 0.0107GLU + 0.011ALA + 0.0148PRO ⟶ mAb
Reaction 9 :	GLN ⟶ GLU + NH_3

Balance for mammalian cell growth can be describes as an “equation” by considering the major “input” and “output” for the biomass formation. The composition of cells can be written as a “molecular formula” and an example for the equation for hybridoma growth is as Equation 2.1 (S. H. Wei, 2004).

$C_6H_{12}C_6$ (glucose) + $0.15C_{6.14}H_{12.36}N_{1.50}O_{2.08}$ (weighted average of amino acids) + $0.34C_5H_{10}N_2O_2$ (glutamine) + $1.39O_2 \longrightarrow 2.37CH_{1.97}N_{0.26}O_{0.49}$ (cell mass) + $0.0058CH_{1.83}N_{0.14}O_{2.06}$ (antibody) + $1.54CO_2 + 1.28H_2O + 1.44C_3H_6O_3$ (lactate) + $0.16NH_3$ (ammonia) + $0.13\ C_4H_7NO_2$ (alanine)

Equation 2.1: Stoichiometric equation for Hybridoma (S. H. Wei, 2004).

Y.H. Guan and R.B. Kemp (1999) had developed a stoichiometric equation for hybridoma growth reaction to represent the metabolic activity. In their study, it was shown that the set of stoichiometric coefficients constitute a validated growth equation has a one-to-one corresponding relationship to the metabolic activity of the average cell population.for both theoretically and experimentally. Table 2.2 is one of the growth reactions for discrete times under activated and triggered condition.

Table 2.2: Stoichiometric equation of hybridoma for discrete times under activated and triggered condition (Y.H. Guan and R.B. Kemp, 1999).

Metabolic condition		**Metabolic reaction**
Activated cells	Catabolism	$C_6H_{12}O_6 + 0.347C_5H_{10}N_2O_3 + 1.7960O_2 \longrightarrow 1.921C_3H_6O_3 + 1.969CO_2 + 0.693NH_3 + 0.929H_2O$
	Anabolism	$0.078C_6H_{12}O_6 + 0.210C_5H_{10}N_2O_3 + 0.149CO_2 \longrightarrow 1.667CH_{1.821}O_{0.837}N_{0.252}$
	Metabolism	$C_6H_{12}O_6 + 0.517C_5H_{10}N_2O_3 + 1.666O_2 + 0.149CO_2 \longrightarrow 1.782C_3H_6O_3 + 1.827CO_2 + 0.693NH_3 + 0.862H_2O + 1.546CH_{1.821}O_{0.837}N_{0.252}$
Triggered cells	Catabolism	$C_6H_{12}O_6 + 0.317C_5H_{10}N_2O_3 + 3.836O_2 \longrightarrow 1.196C_3H_6O_3 + 3.994CO_2 + 0.633NH_3 + 3.044H_2O$
	Anabolism	$0.184C_6H_{12}O_6 + 0.092C_5H_{10}N_2O_3 + 0.054CO_2 \longrightarrow 1.614CH_{1.938}O_{0.922}N_{0.114}$
	Metabolism	$C_6H_{12}O_6 + 0.345C_5H_{10}N_2O_3 + 3.240O_2 \longrightarrow 1.010C_3H_6O_3 + 3.327CO_2 + 0.535NH_3 + 2.571H_2O + 1.363CH_{1.938}O_{0.922}N_{0.114}$

Commercial success of mAb had led to the need for large scale production in mammalian cell culture. Rapid expansion had increase the bioreactor size and optimization effort are improved since then for cell expression and process optimization like fed-batch cultures (J. R. Birch and A. J. Racher, 2006). K. H. Ting and K. A. McDonald (2009) had stated that bioreactor designs must provide an environment that is able to optimize the growth and productivity of the genetically engineered host cells and design of effective bioreactor should consider growth, nutrient uptake and production kinetics, oxygen and heat transfer, and fluid hydrodynamics.

Stirred tank bioreactor have been widely used for commercially antibody production by using cell lines like CHO, hybridoma and NSO and the agitation rates in these reactors are generally kept between 10 to 40 rpm depending upon the sensitivity of the cell lines being cultured (E. Jain and A. Kumar, 2008). L. Legazpi *et al*. (2005) had conducted a study of agitation effect to the hybridoma cell by using rocker set-up and shown high value of specific death rate provoked decreasing in number of viable cell.

Agitation and aeration are critical consideration in bioreactor for mammalian cell because it caused physical cell damage by induced hydrodynamic shear by agitation and bubble damage caused by mass transfer gas sparging (D. M. Marks, 2003).

2.2 Downstream Process

After the upstream section or the fermentation process, the production of mAb is further proceed to downstream section to be purified according to the product quality requirement and there are typical substances that need to be separated in downstream section. Cell culture medium like amino acid, inorganic salts and medium supplement like bovine serum and proteins need to be separated in downstream process. Other than that, substances like intact cells, cell debris, host cell protein and DNA are also separated in order to have pure desired product (S. Sommerfeld and J. Strube, 2005).

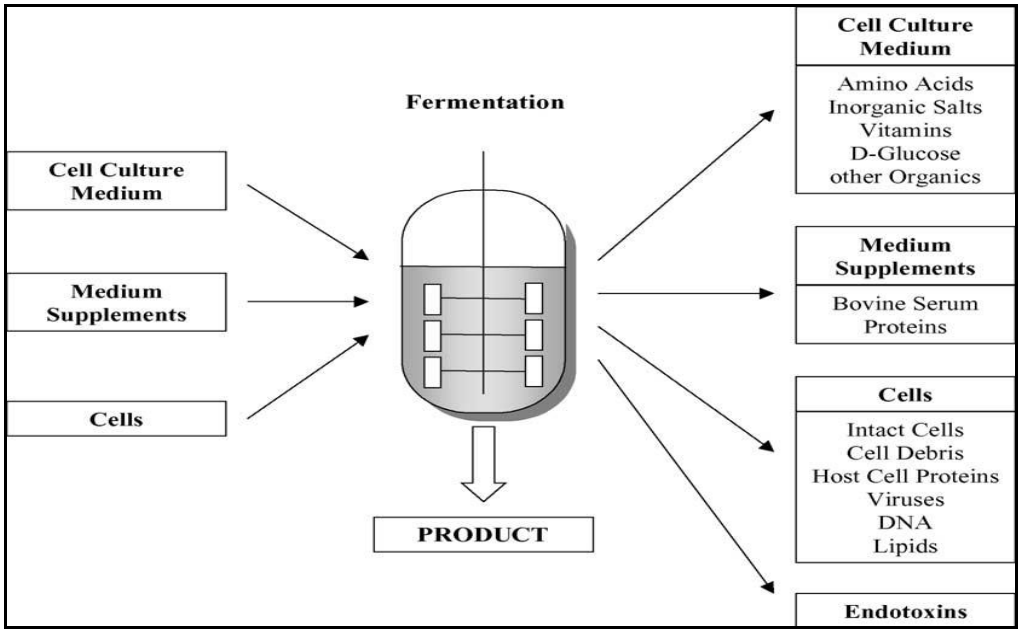

Figure 2.3: Downstream processing task (S. Sommerfeld and J. Strube, 2005).

As known today, mAb is used in many applications such as diagnosis and treatment of certain diseases like cancer therapy. Therapeutic treatment for cancer by using mAb produced by murine hybridomas cultured in a cell bioreactor in serum free conditions were harvested from supernatants and further purified by downstream process (A. L. Horenstein *et al.*, 2003).

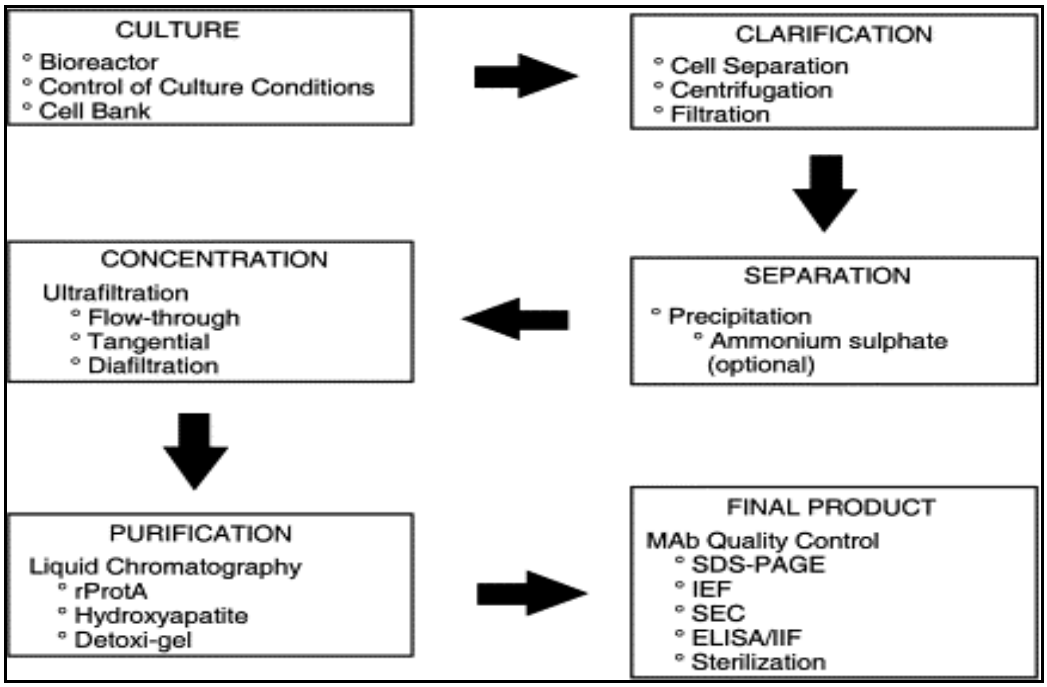

Figure 2.4: Major stages of the downstream processing of mAb for cancer therapy (A. L. Horenstein *et al*., 2003).

Limits imposed by the technology, equipment and facilities that are available had made downstream processes to take proportional response to the limits. As a result, manufacturers are intent to explore several ways or strategies of streamlining product recovery and purification like decreasing the number of steps, avoiding complex steps and reducing raw materials costs. In addition, alternative formats for recovery and purification unit operations are being reconsidered include expanded and simulated moving beds, membrane chromatography and non-chromatographic methods such as flocculation, precipitation, crystallization and aqueous two-phase systems (D. Low *et al*., 2006).

A. A. Shukla *et al*. (2006) had described a flexible, generic platform for mAb downstream processing that they develop at Amgen and applied for the production of over 20 molecules over a range of scales ranging from clinical production to commercial launch.

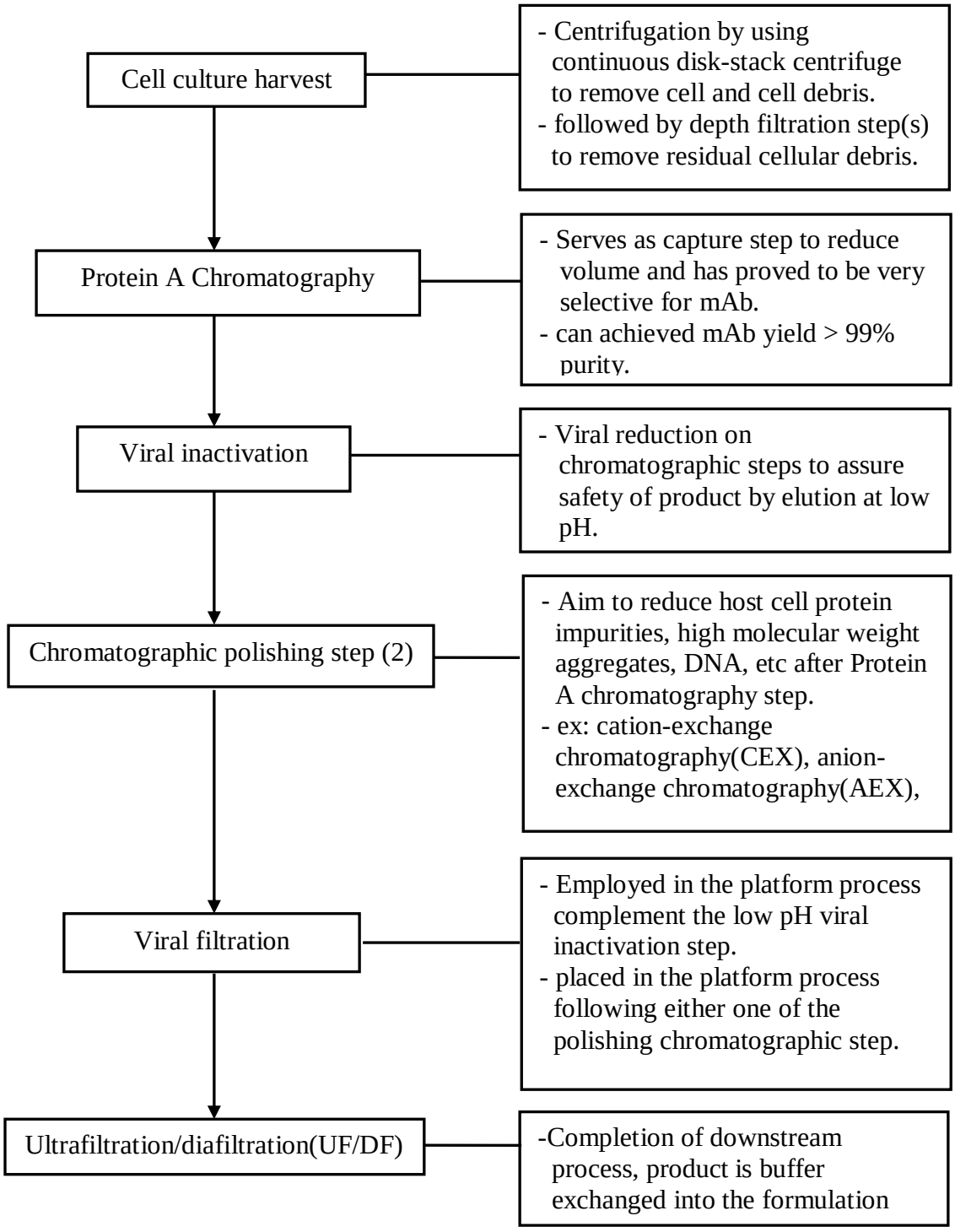

Figure 2.5: Generic Downstream process for mAb (A. A. Shukla *et al*., 2006)

2.2.1 RIPP Scheme

In a broader term, bioseparations engineering refers to the systematic study of the scientific and engineering principles utilized for the large scale purification of biological products. Bioseparation processes are based on multiple techniques and RIPP scheme is commonly used in bioseparation. RIPP stand for Recovery, Isolation, Purification and Polishing that involves use of low resolution techniques first for recovery and isolation followed by high resolution techniques for purification and polishing (R. Ghosh, 2006).

Table 2.3: RIPP scheme for downstream process (R. Ghosh, 2006).

Stage	Objectives	Typical unit operations
Recovery (separation of insoluble)	- Remove or collect cells, cell debris - Reduce volume	Filtration, sedimentation, extraction, adsorption, centrifugation
Isolation	- Remove materials having properties widely different from those target product - Reduce volume	Extraction, adsorption, ultrafiltration, precipitation
Purification	- Remove remaining impurities, which typically are similar to those or target product	Chromatography, affinity methods, precipitation
Polishing	- Remove liquids - Convert product to crystalline form (not always possible)	Drying, crystallization

The objective of RIPP scheme is mainly to reduce volume from the fermentation process until get the pure biological product. The first step that is the recovery step is to remove or collect the cells and cell debris by some unit operations like filtration, sedimentation and centrifugation. Second step that is the isolation step is to remove the

materials that having widely different properties form the target product. This can be done by using extraction, adsorption, precipitation and ultrafiltration.

The next step is purification step that remove remaining impurities that had typical similar type of the target product. This step is very important for downstream process and can be done by using chromatography, affinity and precipitation. The final step is polishing where it is not always possible in downstream process. The objective of this step is to remove liquid and convert the target product into crystalline form by drying and crystallization process.

2.2.2 Centrifugation

Centrifuge is a device that separates particles from suspensions or even macromolecules from solutions according to the size, shape and density by subjecting these dispersed system to artificially induced gravitational fields. After the separation complete, the suspension cells will separate to supernatant and precipitate according to the density of substance in the centrifugation. A disc stack centrifuge is a special type of preparative centrifuge which is compact in design and gives better solid-liquid separation than the standard tubular bowl centrifuge (R. Ghosh, 2006).

For mAb production, centrifugation is the first unit operation that acts as cell removal at the capture step of mAb purification before further process in downstream section (S. Sommerfeld and J. Strube, 2005).